# Plan Overview

*A Data Management Plan created using DMPTool*

**Title:** Molecular and functional study of the movement of urea, water and ammonia (NH3/NH4+) through the urea transporters expressed in Lithobathes catesbeianus oocytes

**Creator:** Raif Musa-Aziz

**Affiliation:** Universidade de São Paulo (www5.usp.br)

**Data Manager:** Jessica Kabutomori Rosa, Neydiana Belize de Pina-Lopes

**Funder:** São Paulo Research Foundation (fapesp.br)

**Funding opportunity number:** 2018/22855-1

**Template:** Digital Curation Centre

**Project abstract:**

Tissues throughout the body generate ammonia as a result of protein degradation. Upon entering circulation, this ammonia travels to the liver, where it is converted into less toxic nitrogenous compounds, urea and glutamine, which can then enter the circulation and travel to the kidneys to be excreted in the urine. In the kidney, urea contributes to maintain a high osmolarity gradient in the medullary interstitium-which is important for the urinary concentrating mechanism-and glutamine is used by the proximal tubule cells to regenerate ammonia in a metabolic process that generates equimolar concentration of new HCO3--which is important to maintain acid-base homeostasis. Ammonia, as NH4+, is then secreted into the proximal fluid, reabsorbed by the thick ascending limb of the Henles loop, and secreted by the collecting duct (CD) through parallel NH3 and H+ transport, which combine and re-form NH4+ that will then be excreted. Recent observations, from our laboratory and others, indicated that the basolateral and apical membranes of CD have low NH3 permeability, suggesting that membrane proteins are likely involved in NH3 (dissolved gas) transport in the CD cell. Mammalian urea transporters (UTs) belong to the SLC14 family of solute carriers and are responsible for the facilitated diffusion of urea across plasma membrane. There are two UT genes: SLC14A1, which encodes UT-B, and SLC14A2, which generates splice variants UT-A1, A2 and A3. UT-B is highly expressed in the red blood cell membrane, and is also localized in endothelial cells of the descending vasa recta and liver. UT-A1 and A3 are expressed, respectively, on the apical and basolateral membranes of the inner medullary CD. UT-A2 is expressed in the thin descending limb of the Henle´s loop. These transporters recycle and thereby concentrate urea in the renal medulla to maintain hyperosmolar interstitium, which is necessary for maximal urine concentration, and also allow the excretion of urea with a minimal volume of water. However, there is some controversy over whether or not UTs are also physiologically relevant for H2O (during dehydration) and NH3 (during acidosis) transport in the kidney. Previous work has shown that mammalian UT-B, heterologously expressed in Xenopus laevis oocytes, not only transport urea but are also capable of transporting H2O and NH3. The crystal structure of the bovine UT-B show that UTs are homotrimeric, proteins similar to the homotetrameric structure of AQP1. Each

monomer consists of two protomers (6 helices each) that fold together to form a hydrophilic urea channel. The N- and C-termini of each monomer are located in the cytosol. At the center of the three monomers is a hydrophobic pore, blocked by lipid molecules. We hypothesize that UT-As can, in addition to urea, transport H2O and NH3, using the monomeric urea channel. The present study will measure the urea, H2O, and NH3 permeabilities of mouse (m) UT-As expressed in Lithobates catesbeianus oocytes, a heterologous expression system developed and standardized by our laboratory. Briefly, urea uptake will be monitored using 14C-urea, osmotic water permeability (Pf) will be computed using video microscopy after placing the oocytes in a hypotonic solution to monitor the rate of cell swelling, and NH3 permeability will be measured using a microelectrode with a blunt tip to record the maximum transient change in pH at the surface of the oocyte (DpHS) caused by NH3 influx. We believe that the results from this study will provide valuable insights into the role(s) of UTs-with their permeabilities to urea, water and NH3 are an important nexus for integrating the excretion of nitrogenous wastes, water, and acid. Furthermore, deepening the knowledge of the mechanisms involved in the excretion of water and acid (ammonia) will improve our understanding of basic kidney function, and could potentially aid in the treatment of hydroelectrolytic and acid-base disorders.

**Start date:** 04-30-2019

**End date:** 10-30-2021

**Last modified:** 03-27-2021

**Copyright information:**

## Molecular and functional study of the movement of urea, water and ammonia (NH3/NH4+) through the urea transporters expressed in Lithobathes catesbeianus oocytes

We have been doing surface protein expression and collecting measurements of urea, H2O, and NH3 transport of mouse (m) UT-As expressed in Lithobates catesbeianus oocytes, a heterologous expression system developed and standardized by our laboratory.

The surface expression has been assessed by biotinylation and western blot. The Urea uptake was monitored using 14C-urea. Osmotic water permeability (Pf) has been computed using video microscopy after placing the oocytes in a hypotonic solution to monitor the rate of cell swelling. The index of NH3 permeability has been measured using a microelectrode with a blunt tip to record the maximum transient change in pH at the surface (s) of the oocyte (delta pHS) caused by NH3 influx.

For surface expression we present western blots.

For the Urea uptake (C14 uptake), we create bar graphs demonstrating the urea uptake for each expressed UT.

For the Osmotic Water Permeabitility (Pf), we create bar graphs demonstrating the Pf for each expressed UT.

For the Index of NH3 Permeabitility (Pf), we create bar graphs demonstrating the delta pHs for each expressed UT.

We perform statistical analysis to determine significant differences between groups.

All surgical and experimental procedures involving animals were previously approved by the Committee of Animal Care and Use at the Institute of Biomedical Sciences of the University of Sao Paulo (protocol # 7971160519).

All copyright materials will subject to negotiations with the publisher. Concerning intellectual property rights, these will shared among the Laboratory, University of Sao Paulo and Fapesp.

At the moment, we have sufficient storage for the data being collected. Also, we routinely save all our data in the computers in our laboratory and back them up on external hard drive (Samsung). The students of the lab - mentioned as data manager - have been responsible for backing up and recovering the data. In the event of an incident, the data will be recovered from the device that was not affected.

I, the Principal Investigator of the laboratory, will manage access and security of the data. Due to the fact that our data are stored in two separate locations, not connected to each other, we feel that the risk of a security treat is minimal.

Since each of our project is an extension of the previous project, all of our data is considered to have long term value.

Our long term is to save all our data to a cloud server that we will purchase.

Once all our data are in the cloud, anyone with permission will be able to access them.

All data will be shared with the people working in the laboratory and established collaborators.

I, Raif Musa Aziz (the Principal Investigator), will be responsible.

May be necessary to purchase some cloud storage spaces, but not at the moment.

---